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Intermittent hypoxia suppression of growth hormone and insulin-like growth factor-I in the neonatal rat liver

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ABSTRACT

Objectives: Extremely low gestational age neonates with chronic lung disease requiring oxygen therapy frequently experience fluctuations in arterial oxygen saturation or intermittent hypoxia (IH). These infants are at risk for multi-organ developmental delay, reduced growth, and short stature. The growth hormone (GH)/insulin-like growth factor-I (IGF-1) system, an important hormonal regulator of lipid and carbohydrate metabolism, promotes neonatal growth and development. We tested the hypothesis that increasing episodes of IH delay neonatal growth by influencing the GH/IGF-I axis.

Design: Newborn rats were exposed to 2, 4, 6, 8, 10, or 12 hypoxic episodes (12% O_2) during hyperoxia (50% O_2) from P0-P7, P0-P14 (IH), or allowed to recover from P7-P21 or P14-P21 (IHR) in room air (RA). RA littermates at P7, P14, and P21 served as RA controls; and groups exposed to hyperoxia only (50% O_2) served as zero IH controls. Histopathology of the liver; hepatic levels of GH, GHBP, IGF-I, IGFBP-3, and leptin; and immunoreactivities of GH, GHR, IGF-I and IGF-IR were determined.

Results: Pathological findings of the liver, including cellular swelling, steatosis, necrosis and focal sinusoid congestion were seen in IH, and were particularly severe in the P7 animals. Hepatic GH levels were significantly suppressed in the IH groups exposed to 6–12 hypoxic episodes per day and were not normalized during IHR. Deficits in the GH levels were associated with reduced body length and increase body weight during IHR suggesting increased adiposity and catchup fat. Catchup fat was also associated with elevations in GHBP, IGF-I, leptin.

Conclusions: IH significantly impairs hepatic GH/IGF-1 signaling during the first few weeks of life, which is likely responsible for hepatic GH resistance, increased body fat, and hepatic steatosis. These hormonal perturbations may contribute to long-term organ and body growth impairment, and metabolic dysfunction in preterm infants experiencing frequent IH and/or apneic episodes.

1. Introduction

Preterm birth, defined as birth before 37 weeks gestation, is a major cause of death and significant morbidities in children [1,2]. Global studies show that approximately 10% of all newborns are born premature [3,4], with substantial developmental disorders and chronic diseases occurring in later life [5–7]. Surviving preterm neonates with immature respiratory control mechanisms experience frequent arterial oxygen desaturations, intermittent hypoxia (IH), apnea of prematurity (AOP) with bradycardia, or tissue injuries associated with recovery/re-oxygenation in hyperoxia or room air [8]. Recurrent IH episodes increases reactive oxygen species (ROS) and oxidative stress, which are implicated in the development of major neonatal co-morbidities which are often the cause of feeding intolerance, poor growth and nutrition, long-term cardiovascular health, and metabolic syndrome in adult life [9–11].

The growth hormone (GH)/insulin-like growth factor (IGF)-I system, which consists of GH, GH receptor (GHR), IGF-I, IGF-II, IGF-I receptor (IGF-IR), and IGF binding proteins (IGFBPs 1–6), plays a critical role in fetal and postnatal growth and development. In the fetus,

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GH levels are high, but it plays a minimal role in fetal growth. This is due to low fetal levels of GH receptor (GHR) and GH binding protein (GHBP), suggesting that fetal growth is independent of GH [12]. Instead, fetal GH regulates carbohydrate metabolism. Immediately after birth, GH levels rapidly decline possibly due to postnatal activation of the GHR and GHBPs [13]. GH is produced by the anterior pituitary gland and is an important regulator of linear growth, metabolism, and body composition from childhood to adult life [14]. It travels through the circulation to target cells and tissues that express its receptor [15]. Although the liver is the major target organ for GH where it has plays a key role in lipid metabolism and glucose homeostasis [16], it regulates cell growth and metabolism in various non-hepatic tissues. For example, in adipose tissue GH stimulates lipolysis; in skeletal muscle it promotes growth, maintenance, repair and regeneration; in bone it promotes longitudinal growth [15]; and the GH/IGF system is involved in renal development and nephron size [17].

GH promotes postnatal somatic growth by inducing the production of IGF-I in the liver [18] and in contrast to GH, is the main regulator of fetal and postnatal growth. IGF-1, a 70-amino acid polypeptide hormone, accounts for about 75% of all circulating IGFs [19], and together with its receptor, is expressed in almost all tissues for autocrine/paracrine purposes [20]. IGF-I expression in the liver markedly exceeds that in any other tissue, and most circulating IGF-1 originates from liver [21]. Regardless, studies have shown that liver-derived IGF-I is not required for postnatal growth, suggesting that local production of IGF-I may be more important than liver-derived circulating IGF-I for body growth [22].

IGF-1 availability is tightly regulated by its binding proteins (IGFBPs), which increase IGF-1 half-life from minutes to hours, and shuttles IGF-I to specific target tissues [23]. IGF-1 deficiency is associated to GH resistance, and its replacement therapy restores altered GH/IGF-1 axis by reducing circulating GH levels [19]. IGF-I is present in high concentrations in serum, and is mostly protein bound [24]. Approximately 90% of IGF-1 is bound to IGFBP-3, the primary hepatic-derived IGFBP [25], which serves as its major constitutive binding protein [26,27]. In mice, 70–80% of IGF-I exists in the circulation as ternary complex consisting of IGF-I, IGFBP-3, and the liver-derived acid-labile subunit (ALS). This ternary complex has a relatively long half-life of 10–16 h [28,29], and may be regulated by GH [30]. In rats, the fetal serum profile, characterized by high IGF-II and IGFBP-2, is replaced around the third week of life by the adult-type profile of high IGF-I and IGFBP-3, with a dramatic reduction in IGF-II and IGFBP-2 [31]. Studies by Ibañez de Cáceres et al. [32] showed that administration of recombinant human GH to rats resulted in improved body weight gain and dose-dependent elevations in serum GHBP-3 compared to other IGFBPs, demonstrating a GH association/dependence of IGFBP-3. The smaller binary complexes of IGF-I and serum IGFBPs (mainly IGFBP-3) comprise 15–20% of the circulating pool, and the remainder (free IGF-I) comprise < 5% with an extremely short half-life [33]. Leptin is a hormone produced by adipocytes that regulates metabolism and appetite. It regulates food intake and body weight and its levels are high in obesity [34]. In mice, leptin deficiency and lack of functional leptin receptors were associated with decreased muscle and bone mass, as well as IGF-1 deficiency, and leptin treatment stimulated the GH/IGF-I axis [35].

The liver is a prominent organ in neonates. It constitutes approximately 5% of body weight at birth (compared to 2% in adults) with a large volume (per unit of body weight) of 48 mL/Kg [36]. Premature neonates have compromised serum IGF-1 levels which contribute in part to poor postnatal growth [37,38]. Intrinsically low levels of IGF-I combined with supplemental oxygen and IH predisposes the preterm liver to increased production of reactive oxygen species (ROS) and oxidative stress. Propagation of ROS that target the liver due to its high lipid content, will result in lipid peroxidation [39–41]. Studies have shown that IH significantly influences the modulation of hepatic GH and IGF-I [42,43]. These alterations can have a negative long-term impact on growth and carbohydrate metabolism. Furthermore, reductions in GH and IGF-I can further propagate ROS accumulation in the liver, particularly in transfused preterm infants with iron deficiency and compromised antioxidant systems. Studies have shown that free iron, via the Fenton reaction reacts with H_2O_2 to induce lipid peroxidation, an effect that can be ameliorated with GH and IGF-I [44,45]. We therefore conducted a series of experiments to understand the role of increasing IH episodes on hepatic GH and IGF-I levels. We hypothesized that there is a critical number of IH episodes which will induce hepatocyte loss, and alter hepatic growth factors. These IH-induced changes will have permanent implications on growth despite recovery and re-oxygenation in room air. To test our hypothesis, we examined GH, GHBP (the soluble form of GHR), and IGF-I, as well as weight accretion in response to increasing IH episodes and during recovery from IH (IHR) in room air.

2. Material and methods

This study was approved by the State University of New York, Downstate Medical Center Animal Care and Use Committee, Brooklyn, NY. Animals were cared for and handled according to the United States Department of Agriculture (USDA) guidelines and National Institutes of Health guide for the Care and Use of Laboratory Animals. Euthanasia was carried out according to the American Veterinary Medical Association Panel for Euthanasia guidelines.

2.1. Experimental design

Certified infection-free, timed-pregnant Sprague Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) at 17 days gestation. The animals were housed in an animal facility with a 12-hour-day/12-hour-night cycle and provided standard laboratory diet and water ad libitum until delivery. Within 2–3 h of birth, newborn pups delivered on the same day were pooled and randomly assigned to expanded litters of 18 pups/l (9 males and 9 females). Gender was determined by the anogenital distance. The expanded litter size was used to simulate relative postnatal malnutrition of critically ill ELGANs. Each pup was weighed and measured for linear growth (crown to rump length in centimeters). A total of 31 groups of 18 rat pups (9 males and 9 females) were studied according to the experimental design previously published [46–49]. The groups are described as follows: 1) Groups 1 to 6 were exposed to 2, 4, 6, 8, 10 or 12 IH cycling episodes from P0 to P7; 2) Groups 7 to 12 were exposed to 2, 4, 6, 8, 10 or 12 IH cycling episodes from P0 to P14; 3) Groups 13 to 18 were exposed to 2, 4, 6, 8, 10 or 12 IH cycling episodes from P0 to P7, followed by re-oxygenation in room air (RA) for 14 days from P7 to P21; 4) Groups 18 to 24 were exposed to 2, 4, 6, 8, 10 or 12 IH cycling episodes from P0 to P14, followed by re-oxygenation in RA for 7 days from P14 to P21; 5) Groups 25 to 28 were exposed to hyperoxia (Hx) consisting of 50% O_2 only for 7 days, 14 days, 7 days with 14 days of re-oxygenation in RA, or 14 days with 7 days of re-oxygenation in RA. These groups served as “0” IH such that the range of IH episodes was 0 to 12; and 6) Groups 29–31 were littermates raised in RA from birth to P7, P14, or P21 with all conditions identical except for atmospheric oxygen and served as RA controls. Animals were weighed at birth (P0) prior to placement in the various oxygen environments, and at P7, P14 and P21. Body length (crown to rump length, cm) was determined simultaneously. Percentage changes in body weight and body length were calculated as weight or length at the end of the experiment (P7, P14 or P21) minus weight or length at birth (P0) divided by the weight or length at birth × 100.

2.2. Intermittent hypoxia (IH) cycling

The IH cycles consisted of hyperoxia (50% O_2)/hypoxia (12% O_2) in stepwise increments of brief (1 min), hypoxia (12%) clusters (3 clusters) during 50% O_2 for a total of 2, 4, 6, 8, 10 or 12 episodes/day. This